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<b>(54) Title:</b> NOVEL THERAPIES FOR CYSTIC KIDNEY DISEASE  <b>(57) Abstract</b>  Methods for treating cystic kidney disease are described. The method includes administering to a subject having, or susceptible to, the disease, an effective amount of a renal therapeutic agent or polynucleotide encoding the protein. Renal therapeutic agents are isolated, renal therapeutic molecules that preferably are selected from a member of an osteogenic protein/bone morphogenic protein (OP/BMP) family within a transforming growth factor-beta superfamily of proteins. A particularly preferred agent has at least 60 % amino acid sequence homology with the group consisting of SEQ ID NOS: 1-5.		

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## NOVEL THERAPIES FOR CYSTIC KIDNEY DISEASE

### Field of the Invention

The present invention relates to the treatment of cystic renal disease in mammalian subjects. The methods involve administering, to patients with cystic renal disease, certain proteins of the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins.

### BACKGROUND OF THE INVENTION

Renal disease affects millions of people worldwide each year and adds substantially to health care costs. In the case of renal organ loss, treatment is usually effected by transplanting organs from donors, by surgical reconstruction, or with mechanical devices. Each of these remedies has shortcomings. Transplantation is limited by donor shortage, surgical reconstruction can create other long-term problems, and mechanical devices cannot perform all the functions of a single organ, and therefore cannot prevent progressive deterioration.

Cystic renal disease, of various presentations and etiologies, is of high prevalence, and causes much morbidity and mortality.

Autosomal recessive ("infantile") polycystic disease is characterized by the renal distal tubules and collecting ducts being dilated into cysts, making the kidneys huge, white, and smooth-surfaced. This is normally fatal in infancy or early childhood; typically, the enormous kidneys restrict the ability the lungs and gut to function. Many children also have congenital portal fibrosis of the liver. Multicystic dysplastic kidney, or "renal dysplasia", is characterized by multiple cysts, usually unilateral. Uremic medullary cystic disease, or "nephronophthisis", refers to a group of diseases (including an "autoimmune familial syndrome": Am. J. Kid. Dis. 10: 389, 1987), with cysts at the cortico-medullary junction and severe damage to the cortex. The gene for the common recessive version was mapped to a basement-membrane protein (Am. J. Hum. Genet. 53:1256, 1993).

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic disease, affecting about 500,000 people in the United States, and an estimated 5 to 10 million people worldwide. About 5-10% of all renal transplant and dialysis

patients have this disorder. The cost of treatment, dialysis, and kidney transplantation related to ADPKD exceeds \$1 billion each year.

ADPKD usually does not symptomatically present until adult life. Hundreds of cysts, measuring up to 4 cm in diameter, develop from all levels of the nephron, including Bowman's capsule. As the cysts form, the surrounding normal kidney cells undergo apoptosis (N. Engl. J. Med. 333: 18 & 56, 1995). By the time the patient is forty years old, the kidneys are often the size of footballs. Surprisingly, they may still be working. Half of these patients are on dialysis or transplanted by the age of 70. Patients typically get high blood pressure as adults, years before renal failure develops. Many die of ruptured berry aneurysms. The disease has complete penetrance.

In addition to renal failure, ADPKD is associated with a number of other complications. About 60% of patients have back, flank, or abdominal pain that can be severe and disabling. Other complications of the disease include hematuria, kidney stones, hypertension, urinary infections, and development of cysts in the liver, spleen, pancreas, lungs, gonads, thyroid, uterus and bladder. One tenth of patients die from an intracranial aneurism, possibly related to the hypertension. ADPKD is diagnosed by soundwave testing (ultrasound), radioactive scanning, or x-ray dye testing (intravenous pyelography or IVP). Recently testing for the associated gene has been described (Lancet 2: 6, 1986; Am. J. Kid. Dis. 13: 85, 1989). Treatment is aimed at complications, such as high blood pressure, pain, or infection of the cysts. The disease itself has no cure.

Autosomal dominant polycystic kidney disease is caused by mutations in at least three different genes: PKD1, PKD2, and PKD3, with the implicated mutation on chromosome 16p1.3 (PCKD 1 locus) where it codes for "polycystin", a tubular organizer (Proc. Natl. Acad. Sci. 93: 1524, 1996) Polycystin, a 460 kd protein with a host of domains implicating a potential role in cell-cell and cell-matrix regulation, is encoded by a 52 kb gene with a 14 kb mRNA. The PKD2 protein is also large (110 kd) and is thought to interact with polycystin. ADPKD is caused by mutated DNA that encodes an abnormal form of polycystin. Polycystin appears to have a normal role in the differentiation of epithelial cells, and when defective, these cells fail to mature fully. These incompletely differentiated cells proliferate abnormally and express altered

amounts of otherwise normal electrolyte transport proteins that result in excessive secretion of solute and fluid into the cysts. The proliferation of the cells and the associated apoptosis, and the secretion of the fluid into the cysts created by the enlarging tubule segments appear to be regulated by growth factors, hormones, and cytokines that can alter the extent to which the disease is clinically expressed among individuals. The formation of the cysts is associated with complex changes in the extracellular matrix of the kidneys and other organs that may be directly or indirectly tied to mutated polycystin. The summation of these pathogenetic elements leads to renal interstitial infiltration, with monocytes, macrophages, and fibroblasts culminating in fibrosis and progressive loss of renal function.

### SUMMARY OF THE INVENTION

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects at risk, as defined herein, of cystic kidney disease or the need for renal replacement therapy. Useful renal therapeutic agents include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins, at least a portion of which, exhibit at least 60% amino acid sequence homology with the amino acid sequence of the six- or seven-cysteine domain of human OP-1; and which are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal model of cystic kidney disease, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal at risk of cystic kidney disease.

One aspect of the invention is a method of treating cystic kidney disease comprising administering to a subject having, or at risk for, cystic kidney disease, an

isolated, renal therapeutic agent or an isolated polynucleotide sequence encoding, upon expression, an isolated, renal therapeutic agent. Preferably, the isolated, renal therapeutic agent is selected from a member of an osteogenic protein/bone morphogenic protein (OP/BMP) family within a transforming growth factor-beta superfamily of proteins. Most preferably, the isolated, renal therapeutic agent includes a portion of which has at least 60% amino acid sequence homology with amino acid sequences of SEQ ID NOS: 1-5. Further, the step of administering also includes administering an isolated polypeptide selected from the group consisting of those polypeptides listed in Table 1 and biologically active homologs thereof. Preferred methods include the step of administering via systemic or local administration.

A further method of the invention comprises the step of administering to a subject having, or at risk for, cystic kidney disease, a therapeutically effective composition selected from the group consisting of: (i) an isolated, renal therapeutic agent; (ii) an isolated polynucleotide capable of encoding, upon expression, an isolated renal therapeutic agent; and (iii) an isolated polynucleotide sequence capable of hybridizing under stringent conditions to the isolated polynucleotide sequence of (ii). The diseases treatable by this method include autosomal recessive ("infantile") polycystic disease, multicystic dysplastic kidney disease, uremic medullary cystic disease, and autosomal dominant polycystic kidney disease.

In another aspect of the invention, the method includes administration of a renal therapeutic agent is a polypeptide that is soluble under physiological conditions and in physiological solutions.

The method of the present invention includes prophylactic prevention of a renal condition (i.e., dysfunction) in a mammal at risk for a cystic kidney disease and/or treatment in order to arrest the development and retard the progression of the cystic kidney disease in a mammal having clinical manifestations of the cystic kidney disease. The methods are used for promoting, among other things, the restoration of a functioning renal system. As such they are of great value in preventing or delaying the onset of chronic renal failure, with the resultant need for chronic dialysis or renal replacement therapy. As such, they are useful in prolonging the lives, and in

maintaining the quality of life, of subjects at risk of, or already afflicted with, cystic kidney disease.

### DESCRIPTION OF THE SEQUENCES

5

Human OP1 protein species defining the conserved 7 cysteine skeleton in the active region .

SEQ ID NO:1:

10

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly

15

Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala

Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro Lys

Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe

20

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val

Arg Ala Cys Gly Cys His

25

Human OP1 protein species defining the conserved 6 cysteine skeleton in the active region

30

SEQ ID NO: 2

Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala

35

Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro

Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu

Val His Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro

40

Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn

Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys

His

"Seven cysteine template" (SEQ ID NO: 3):

5	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Cys
10	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
15	Cys	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
20	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Cys	Xaa	Cys	Xaa		

25 "Eight cysteine template" (SEQ ID NO: 4 comprising additional five residues at the N-terminus, including a conserved cysteine residue):

	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
30	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys
	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa
35	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
40	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Cys	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa  
 Cys Xaa

5

SEQ ID NO: 5 is a composite sequence (beginning at residue 38 of Fig. 24 from U.S. Patent 5,266,683), and includes both the specific amino acid sequence created by the amino acid identity shared by the four OP1, OP2 species, as well as alternative residues for the variable positions within the sequence.

10

SEQ ID NO: 5

15	Cys 1	Xaa	Xaa	His	Glu	Leu	Tyr	Val	Ser	Phe
	Xaa	Asp	Leu	Gly	Trp	Xaa	Asp	Trp	Xaa	Ile
	Ala	Pro	Xaa	Gly	Tyr	Xaa	Ala	Tyr	Tyr	Cys
20	Glu	Gly	Glu	Cys	Xaa	Phe	Pro	Leu	Xaa	Ser
	Xaa	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Xaa
	Gln	Xaa	Leu	Val	His	Xaa	Xaa	Xaa	Pro	Xaa
25	Xaa	Val	Pro	Lys	Xaa	Cys	Cys	Ala	Pro	Thr
	Xaa	Leu	Xaa	Ala	Xaa	Ser	Val	Leu	Tyr	Xaa
30	Asp	Xaa	Ser	Xaa	Asn	Val	Ile	Leu	Xaa	Lys
	Xaa	Arg	Asn	Met	Val	Val	Xaa	Ala	Cys	Gly
	Cys	His								

35

and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 = (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res.

40

82 = (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 89 = (Lys or Arg);  
Xaa at res. 91 = (Tyr or His); and Xaa at res. 97 = (Arg or Lys).

5

## DETAILED DESCRIPTION OF THE INVENTION

All references cited in the detailed description are, unless otherwise stipulated, incorporated herein by reference. None of the references cited in the Background are admitted to be prior art.

### 10 I. Definitions

The invention will now be described with reference to the following detailed description of which the following definitions are included:

15 "Cystic kidney disease" includes any disease characterized by the presence of cysts in the kidney. The group specifically includes, but is not limited to, autosomal recessive ("infantile") polycystic disease, multicystic dysplastic kidney, uremic medullary cystic disease, and autosomal dominant polycystic kidney disease. The presence of such a disease can be made on the basis of clinical observations, or by genetic testing for implicated genes.

20

"Renal therapeutic agent" means a polypeptide, or a functional variant of a polypeptide, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins, at least a portion of which exhibit  
25 at least 60% amino acid sequence homology with the amino acid sequence of the six- or seven-cysteine domain of human OP-1; and which is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive  
30 loss of renal function in a standard animal model of cystic kidney disease, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal at risk of cystic kidney disease. As used

herein, a percentage "homology" between two amino acid sequences indicates the percentage of amino acid residues which are identical or similar between the sequences and, as used herein, "similar" residues are "conservative substitutions" which fulfill the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C. Such proteins are defined in Section IIB, below.

"Prophylactic efficacy" of a renal therapeutic agent of the invention and an amount of the agent is said to be "prophylactically effective," if administration of that amount of the agent is sufficient to cause a significant increase in the average patient age at which the disease progresses to the point at which the patient's GFR is 50% or less of normal, at which time the patient's disease is considered to be "clinically significant."

"Therapeutic efficacy" of a renal therapeutic agent of the invention, and an amount of the agent is said to be "therapeutically effective," if administration of that amount of the agent is sufficient to cause a clinically significant improvement in a standard marker of renal function when administered to a human patient who has been diagnosed with cystic renal disease. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na+), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like. See, for example, Brenner and Lazarus in **Harrison's Principles of Internal Medicine**, 13th ed., Isselbacher et al., (eds), McGraw Hill Text, NY.

As used herein, a subject is said to be "at risk" of developing clinically significant cystic renal disease if the subject is reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephron units associated with cystic renal disease. Subjects at risk of cystic renal

disease include those having a demonstrated mutation in a gene associated with developing cystic kidney disease, and also having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected GFR for the subject.

5

"amino acid"- a monomeric unit of a peptide, polypeptide, or protein. There are twenty L-isomers of amino acids. The term also includes analogs of the amino acids and D-isomers of the protein amino acids and their analogs.

10 "protein"- any polymer consisting essentially of any of the 20 protein amino acids, regardless of its size. Although "polypeptide" is often used in reference to relatively large polypeptides, and "peptide" is often used in reference to small polypeptides, usage of these terms in the art overlaps and is varied. The term "protein" as used herein refers to peptides, proteins and polypeptides, unless otherwise noted.

15 "genetic fusion"- refers to a co-linear, covalent linkage of two or more proteins via their individual peptide backbones, through genetic expression of a polynucleotide molecule encoding those proteins.

20 "mutant" - any change in quantity or structure of genetic material of an organism, in particular any change (i.e., deletion, substitution, addition, or alteration) in a wild type renal therapeutic agent polynucleotide sequence or any change in a wild type renal therapeutic agent protein.

25 "wild-type" - the naturally-occurring polynucleotide sequence of a renal therapeutic agent exon, or a portion thereof, or renal therapeutic agent protein, or portion thereof, respectively, as it exists in vivo.

30 "standard hybridization conditions"- salt and temperature conditions substantially equivalent to 0.5 X SSC to about 5 X SSC and 65 degrees C for both hybridization and wash. The term "standard hybridization conditions" as used herein is therefore an operational definition and encompasses a range of hybridization. Higher

stringency conditions may, for example, include hybridizing with plaque screen buffer (0.2% polyvinylpyrrolidone, 0.2% Ficoll 400; 0.2% bovine serum albumin, 50 mM Tris-HCl (pH 7.5); 1 M NaCl; 0.1% sodium pyrophosphate; 1 % SDS); 10% dextran sulphate, and 100 ug/ml denatured, sonicated salmon sperm DNA at 65 degrees C for 12-20 hours, and washing with 75 mM NaCl/7.5 mM sodium citrate (0.5 x SSC)/1% SDS at 65 degrees C. Lower stringency conditions may, for example, include hybridizing with plaque screen buffer, 10% dextran sulphate and 110 ug/ml denatured, sonicated salmon sperm DNA at 55 degrees C for 12-20 hours, and washing with 300 mM NaCl/30mM sodium citrate (2.0 X SSC)/1% SDS at 55 degrees C. See also

10 **Current Protocols in Molecular Biology**, John Wiley & Sons, Inc. New York, Sections 6.3.1-6.3.6, (1989).

"expression control sequence"- a sequence of polynucleotides that controls and regulates expression of genes when operatively linked to those genes.

15

"operatively linked"- a polynucleotide sequence (DNA, RNA) is operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the polynucleotide sequence to be expressed and maintaining the correct reading frame to permit expression of the polynucleotide sequence under the control of the expression control sequence and production of the desired renal therapeutic polypeptide encoded by the polynucleotide sequence.

25 "expression vector"- a polynucleotide, most commonly a DNA plasmid, which allows expression of at least one gene when the expression vector is introduced into a host cell. The vector may, or may not, be able to replicate in a cell.

"Isolated" (used interchangeably with "substantially pure"), when applied to nucleic acid i.e., polynucleotide sequences, that encode renal therapeutic agent polypeptides, means an RNA or DNA polynucleotide, portion of genomic

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polynucleotide, cDNA or synthetic polynucleotide which, by virtue of its origin or manipulation: (i) is not associated with all of a polynucleotide with which it is associated in nature (e.g., is present in a host cell as an expression vector, or a portion thereof); or (ii) is linked to a nucleic acid or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature. By "isolated" it is further meant a polynucleotide sequence: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) chemically synthesized; (iii) recombinantly produced by cloning; or (iv) purified, as by cleavage and gel separation. Thus, "substantially pure nucleic acid" is a nucleic acid which is not immediately contiguous with one or both of the coding sequences with which it is normally contiguous in the naturally occurring genome of the organism from which the nucleic acid is derived. Substantially pure DNA also includes a recombinant DNA which is part of a hybrid gene encoding additional renal therapeutic agent sequences.

"Isolated" (used interchangeably with "substantially pure"), when applied to a renal therapeutic agent polypeptides means a polypeptide or a portion thereof which, by virtue of its origin or manipulation: (i) is present in a host cell as the expression product of a portion of an expression vector; or (ii) is linked to a protein or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature. By "isolated" it is further meant a protein that is : (i) chemically synthesized; or (ii) expressed in a host cell and purified away from associated proteins, as by gel chromatography. The term generally means a polypeptide that has been separated from other proteins, lipids and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from substances such as antibodies or gel matrices (polyacrylamide) which are used to purify it. It is preferred that an isolated polypeptide constitute at least 10 % dry weight of a purified preparation, although it may constitute higher percentages e.g., 20,50,70,80 or 95%. The purified preparation also preferably contains sufficient polypeptide to allow protein sequencing. This weight may vary over a wide range, i.e, from 1 microgram to 100 milligram of the polypeptide

A "heterologous promoter" as used herein is a promoter which is not naturally associated with a gene or a purified nucleic acid.

"Homologous"- as used herein is synonymous with the term "identity" and refers to the sequence similarity between two polypeptides, molecules or between two polynucleotides. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit (for instance, if a position in each of the two DNA molecules is occupied by adenine, or a position in each of two polypeptides is occupied by a lysine), then the respective molecules are homologous at that position. The percentage homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For instance, if 6 of 10 of the positions in two sequences are matched or are homologous, then the two sequences are 60% homologous. By way of example, the DNA sequences CTGACT and CAGGTT share 50% homology (3 of the 6 total positions are matched). Generally, a comparison is made when two sequences are aligned to give maximum homology. Such alignment can be provided using, for instance, the method of Needleman et al., *J. Mol Biol.* 48: 443-453 (1970), implemented conveniently by computer programs such as the Align program (DNASTar, Inc.). Homologous sequences share identical or similar amino acid residues, where similar residues are conservative substitutions for, or "allowed point mutations" of, corresponding amino acid residues in an aligned reference sequence. In this regard, a "conservative substitution" of a residue in a reference sequence are those substitutions that are physically or functionally similar to the corresponding reference residues, e.g., that have a similar size, shape, electric charge, chemical properties, including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation" in Dayhoff et al., 5: **Atlas of Protein Sequence and Structure**, 5: Suppl. 3, chapter 22: 354-352, Nat. Biomed. Res. Foundation, Washington, D.C. (1978).

A "renal therapeutic agent homolog" includes all: (i) polypeptides derived from a renal therapeutic agent protein having similar biological activity to, e.g., OP-1, the

homologs preferably including biologically active variants of mammalian origin; (ii) protein molecules having biological activity produced by screening with a renal therapeutic agent probe; (iii) ligands of renal therapeutic agent polypeptides; (iv) non-polypeptide molecules having the identical biological activity as, for example, OP-1; and (v) all renal therapeutic agent homologs.

The terms "peptide", "proteins" and "polypeptides" are used interchangeably herein. The terms "polynucleotide sequence" and "nucleotide sequence" are also used interchangeably herein. The term "renal therapeutic fragment" is used interchangeably with "renal therapeutic agent".

A "purified preparation of cells" refers to an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and preferably 50% of the subject cells.

The term "transgene" means a nucleic acid sequence encoding one or more polypeptides which is partly or entirely heterologous (i.e., foreign) to the transgenic animal or cell into which it is introduced, or is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted or is inserted into the animal or cell genome in such a way as to alter the genome of the cell into which it is inserted by being inserted at a location which differs from that of the natural gene or which, by its insertion, results in a "knockout". A transgene can include one or more transcriptional regulatory sequences and any other nucleic acids, such as introns, that may be needed for optimal expression of the selected nucleic acids, all operably linked to the selected nucleic acid, and may include an enhancer sequence.

A "transgenic cell" refers to a cell containing a transgene.

A "transgenic animal" is any animal into which one or more and preferably essentially all, of the cells of the animal includes a transgene. The transgene can be

introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by micro injection or by infection with a recombinant virus. The molecule may be integrated within a chromosome or it may be extrachromosally replicating DNA.

5

Practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, **Molecular Cloning: A Laboratory Manual**, 2nd edition. (Sambrook, Fritsch and Maniatis, eds.), Cold Spring Harbor Laboratory Press, 1989; **DNA Cloning**, Volumes I and II (D.N. Glover, ed), 1985; **Oligonucleotide Synthesis**, (M.J. Gait, ed.), 1984; U.S. Patent No. 4,683,195 (Mullis et al.); **Nucleic Acid Hybridization** (B.D. Hames and S.J. Higgins, eds.), 1984; **Transcription and Translation** (B.D. Hames and S.J. Higgins, eds.), 1984; **Culture of Animal Cells** (R.I. Freshney, ed). Alan R. Liss, Inc., 1987; **Immobilized Cells and Enzymes**, IRL Press, 1986; **A Practical Guide to Molecular Cloning** (B. Perbal), 1984; **Methods in Enzymology**, Volumes 154 and 155 (Wu et al., eds), Academic Press, New York; **Gene Transfer Vectors for Mammalian Cells** (J.H. Miller and M.P. Calos, eds.), 1987, Cold Spring Harbor Laboratory; **Immunochemical Methods in Cell and Molecular Biology** (Mayer and Walker, eds.), Academic Press, London, 1987; **Handbook of Experiment Immunology**, Volumes I-IV (D.M. Weir and C.C. Blackwell, eds.), 1986; **Manipulating the Mouse Embryo**, Cold Spring Harbor Laboratory Press, 1986.

25

## **II. Description of the Preferred Embodiments**

### **A. General**

Administration of the renal therapeutic agents of the present invention can prevent, inhibit or delay the development or progression of cystic renal disease, which usually leads to the need for renal replacement therapy (i.e., renal transplant or chronic dialysis) or death. In preferred embodiments, the therapeutic agents of the invention

30

are members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins.

#### B. Renal Therapeutic Agents

5       The renal therapeutic agents of the present invention are naturally occurring proteins, or functional variants of naturally occurring proteins, in the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- $\beta$  superfamily of proteins. That is, these proteins form a distinct subgroup, referred to herein as the "OP/BMP family," within the loose evolutionary grouping of sequence-related proteins  
10       known as the TGF- $\beta$  superfamily. Members of this protein family comprise secreted polypeptides that share common structural features, and that are similarly processed from a pro-protein to yield a carboxy-terminal mature protein. Within the mature protein, all members share a conserved pattern of six or seven cysteine residues defining a 97-106 amino acid domain (see SEQ ID NOS: 1-5), and the active form of  
15       these proteins is either a disulfide-bonded homodimer of a single family member, or a heterodimer of two different members (see, e.g., Massague (1990), Annu. Rev. Cell Biol. 6:597; Sampath et al. (1990), J. Biol. Chem. 265:13198). For example, in its mature, native form, natural-sourced human OP-1 is a glycosylated dimer typically having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE.  
20       When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. The unglycosylated protein has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptide chains, having molecular weights of about 14 kDa to 16 kDa.

25       Typically, the naturally occurring OP/BMP proteins are translated as a precursor, having an N-terminal signal peptide sequence, a "pro" domain, and a "mature" protein domain. The signal peptide is typically less than 30 residues, and is cleaved rapidly upon translation at a cleavage site that can be predicted using the method of Von Heijne (1986), Nucleic Acids Research 14:4683-4691. The "pro"  
30       domain is variable both in sequence and in length, ranging from approximately 200 to over 400 residues. The pro domain is cleaved to yield the "mature" C-terminal domain

of approximately 115-180 residues, which includes the conserved six- or seven-cysteine C-terminal domain of 97-106 residues. As used herein, the "pro form" of an OP/BMP family member refers to a protein comprising a folded pair of polypeptides, each comprising a pro domain in either covalent or noncovalent association with the mature domains of the OP/BMP polypeptide. Typically, the pro form of the protein is more soluble than the mature form under physiological conditions (e.g., in vivo) and in physiological solutions (e.g., biocompatible solutions such as blood, saline or pharmaceutical carriers that are capable of being introduced into a living subject, it being understood by workers of ordinary skill in the art that the biocompatibility of a given solution is not a fixed quantity and may depend in large part upon the dosage.)

The pro form appears to be the primary form secreted from cultured mammalian cells. The "mature form" of the protein refers to mature C-terminal domain which is not associated, either covalently or noncovalently, with the pro domain. Any preparation of OP-1 is considered to contain mature form when the amount of pro domain in the preparation is no more than 5% of the amount of "mature" C-terminal domain.

OP/BMP family members useful herein include any of the known naturally-occurring native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally-sourced or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as new, active members of the OP/BMP family of proteins.

Particularly useful sequences include those comprising the C-terminal seven cysteine domains of mammalian, preferably human, human OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP8 and BMP9. Other proteins useful in the practice of the invention include active forms of GDF-5, GDF-6, GDF-7, DPP, Vg1, Vgr-1, 60A, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP10, BMP11, BMP13, BMP15, and amino acid sequence variants thereof. In one currently preferred embodiment, the renal therapeutic agents of the invention are selected from any one of: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

Publications disclosing these sequences, as well as their chemical and physical properties, include: OP-1 and OP-2: U.S. Patent No. 5,011,691, U.S. Patent

- No. 5,266,683, and Ozkaynak et al. (1990), EMBO J. 9:2085-2093; OP-3: WO94/10203; BMP2, BMP3, and BMP4: U.S. Patent No. 5,013,649, WO91/18098, WO88/00205, and Wozney et al. (1988), Science 242:1528-1534; BMP5 and BMP6: WO90/11366 and Celeste et al. (1991), Proc. Natl. Acad. Sci. (USA) 87:9843-9847;
- 5 Vgr-1: Lyons et al. (1989), Proc. Natl. Acad. Sci. (USA) 86: 4554-4558; DPP: Padgett et al. (1987), Nature 325:81-84; Vgl: Weeks (1987), Cell 51:861-867; BMP-9: WO95/33830; BMP10: WO94/26893; BMP-11: WO94/26892; BMP12: WO95/16035; BMP-13: WO95/16035; GDF-1: WO92/00382 and Lee et al. (1991), Proc. Natl. Acad. Sci. (USA) 88:4250-4254; GDF-8: WO94/21681; GDF-9:
- 10 WO94/15966; GDF-10: WO95/10539; GDF-11: WO96/01845; BMP-15: WO96/36710; MP121: WO96/01316; GDF-5 (CDMP-1, MP52): WO94/15949, WO96/14335, WO93/16099 and Storm et al. (1994), Nature 368:639-643; GDF-6 (CDMP-2, BMP13): WO95/01801, WO96/14335 and WO95/10635; GDF-7 (CDMP-3, BMP12): WO95/10802 and WO95/10635; BMP-3b: Takao, et al. (1996), Biochem.
- 15 Biophys. Res. Comm. 219:656-662; GDF-3: WO94/15965; 60A: Blaster et al. (1993), Cell 73:687-702 and GenBank accession number L12032. In another embodiment, useful proteins include biologically active biosynthetic constructs, including novel biosynthetic proteins and chimeric proteins designed using sequences from two or more known OP/BMP family proteins. See also the biosynthetic constructs disclosed in U.S.
- 20 Patent No. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

In still another preferred aspect, the invention comprises methods of using renal therapeutic proteins encoded by nucleic acids which hybridize to DNA or RNA sequences encoding the above-identified proteins; more particularly which hybridize

25 under standard conditions to DNA and RNA sequences encoding the active region of OP1 or OP2 (see, for example amino acid SEQ ID NOS: 1 -5, herein. The invention further comprises nucleic acids and the therapeutically effective polypeptide chains encoded by these nucleic acids which hybridize to the "pro" region of the OP1 or OP2 proteins under standard hybridization conditions. In another aspect, the invention

30 provides a substantially pure nucleic acid having or comprising a nucleotide sequence which encodes a polypeptide, the amino acid sequence of which includes, or is, the

sequence of a renal therapeutic agent. In preferred embodiments, the subject renal therapeutic nucleic acid will include a transcriptional regulatory sequence, e.g. at least one of a transcriptional promoter or transcriptional enhancer sequence, operably linked to the renal therapeutic gene sequence, e.g., to render the renal therapeutic gene  
5 sequence suitable for use as an expression vector. In yet a further preferred embodiment, the nucleic acid which encodes a renal therapeutic polypeptide of the invention, hybridizes under standard conditions to a nucleic acid probe corresponding to at least 12 consecutive nucleotides from a polynucleotide sequence that encodes, for example, SEQ ID NOS: 1 or 2, more preferably to at least 20 consecutive nucleotides  
10 from a polynucleotide sequence that encodes, for example, SEQ ID NOS: 1 or 2. In a preferred embodiment, the nucleic acid differs by at least one nucleotide from a nucleotide sequence which encodes amino acids 330 to 431 from a region of human OP-1

Particular useful renal therapeutic agents include those listed in Table I. Table  
15 I lists various species of the hOP1 protein and DNA identified to date, including their Sequence Listing references. As described above, in its native form hOP1 expression yields an immature translation product ("hOP1-PP", where "PP" refers to "prepro form") of about 400 amino acids that subsequently is processed to yield a mature sequence of 139 amino acids ("OP1-18Ser".) The "active region" (also called the  
20 "functional domain") of the protein includes the C-terminal 97 amino acids of the OP1 sequence ("OPS": see SEQ ID NO: 2). A longer active sequence is OP7 (comprising the C-terminal 102 amino acids: SEQ ID NO: 1).

Furthermore, analogs of the active region, e.g., non-native forms never before known in nature, designed based on the observed homologies and known structure and  
25 properties of the native protein can be capable of having therapeutic efficacy. The amino acid sequences of the renal therapeutic proteins disclosed herein share significant homology with various regulatory proteins on which a consensus probe can be modeled. In addition, these proteins share a conserved six or seven cysteine skeleton in this region (e.g., the linear arrangement of these C-terminal cysteine residues is  
30 conserved in the different proteins.) See, for example, SEQ ID NO: 1, the sequence which defines the seven cysteine skeleton, or SEQ ID NO: 2, the sequence defines the

six cysteine skeleton. In addition, the OP2 proteins contain an additional cysteine residue within this region.

TABLE I

5	hOP1	DNA sequence encoding human OP1 protein (Seq. ID No. 1 or 3, described in U.S. Patent No. 5,266,683).
10	hOP1-PP	Amino acid sequence of human OP1 protein (prepro form), Seq. ID No. 1, residues 1-431, described in U.S. Patent 5,266,683.
15	OP1-18Ser	Amino acid sequence of mature human OP1 protein, Seq. ID No. 1, residues 293-431. N-terminal amino acid is serine. described in U.S. Patent No. 5,266,683.
20	OPS	Human OP1 protein species defining the conserved 6 cysteine skeleton in the active region (97 amino acids, Seq. ID No. 2, residues 335-431, described in U.S. Patent No. 5,266,683 and as the amino acid residues in SEQ ID NO: 2, herein).
25	OP7	Human OP1 protein species defining the conserved 7 cysteine skeleton in the active region (102 amino acids, Seq. ID No. 2, residues 330-431, described in U.S. Patent 5,266,683 and as SEQ ID NO: 1, herein).
30	OP1-16Ser	N-terminally truncated mature human OP1 protein species. (Seq. ID No. 1, residues 300-431, described in U.S. Patent No. 5,266,683). N-terminal amino acid is serine.
35	OP1-16Leu	N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 313-431, described in U.S. Patent No. 5,266,683. N-terminal amino acid is leucine.
40	OP1-16Met	N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 315-431, described in U.S. Patent No. 5,266,683. N-terminal amino acid is methionine

- OP1-16Ala N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residue 316-431, described in U.S. Patent No. 5,266,683. N-terminal amino acid is alanine
- 5 OP1-16Val N-terminally truncated mature human OP1 protein species, Seq. ID No. 1, residues 318-431, described in U.S. Patent No. 5,266,683. N-terminal amino acid is valine.
- 10 mOP1 DNA encoding mouse OP1 protein, Seq. ID No. 24, described in U.S. Patent No. 5,266,683.
- 15 mOP1-PP Prepro form of mouse protein, Seq. ID No. 24, residues 1-430, described in U.S. Patent No. 5,266,683.
- hOP2 DNA encoding human OP2 protein, Seq. ID No. 28, described in U.S. Patent No. 5,266,683
- 20 hOP2-PP Prepro form of human OP2 protein, Seq. ID No. 28, res. 1-402, described in U.S. Patent No. 5,266,683.
- 25 hOP2-Ala Possible mature human OP2 protein species: Seq. ID No. 28, residues 264-402, described in U.S. Patent No. 5,266,683.
- 30 hOP2-Pro Possible mature human OP2 protein species: Seq. ID NO. 28, residues 267-402, described in U.S. Patent No. 5,266,683. N-terminal amino acid is proline.
- 35 hOP2-Arg Possible mature human OP2 protein species: Seq. ID No. 28, res. 270-402, described in U.S. Patent No. 5,266,683. N-terminal amino acid is arginine.
- 40 hOP2-Ser Possible mature human OP2 protein species: Seq. ID No. 28, res. 243-402, described in U.S. Patent No. 5,266,683. N-terminal amino acid is serine.

In one preferred aspect, the invention comprises methods using renal therapeutic  
45 proteins wherein the proteins comprise a polypeptide chain having an amino acid

sequence sufficiently duplicative of the encoded amino acid sequence of the Seq No: 1 (hOP1) that described in U.S. Patent No. 5,266,683 or the Seq No: 28 (hOP2) that is also described in U.S. Patent No. 5,266,683. As used herein, the term "sufficiently duplicative" is understood to encompass all renal therapeutic agents whose amino acid  
5 sequence comprises at least the conserved six- or seven- cysteine skeleton and shares greater than 60% amino acid sequence identity in its active region with SEQ ID NO: 1 (seven cysteine region) and SEQ ID NO: 2 (six cysteine region) described herein.

## SEQ ID NO: 1:

10

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly

15

Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala

Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro Lys

20

Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val

Arg Ala Cys Gly Cys His

25

## SEQ ID NO: 2

30

Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala

Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro

Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu

35

Val His Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro

Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn

40

Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys

His

A preferred generic amino acid sequence useful as a subunit of a renal therapeutic agent capable of having therapeutic efficacy, and which incorporates the maximum homology between identified OP/BMP proteins can be described by the sequence referred to herein as SEQ ID NO: 5, described below and also to be found as Seq No: 30, in U.S. Patent 5,266,683. SEQ ID NO: 5 is a composite sequence (beginning at residue 38 of Fig. 24 from U.S. Patent 5,266,683), and includes both the specific amino acid sequence created by the amino acid identity shared by the four OP1, OP2 species, as well as alternative residues for the variable positions within the sequence.

## SEQ ID NO: 5

15	Cys	Xaa	Xaa	His	Glu	Leu	Tyr	Val	Ser	Phe
	1									
	Xaa	Asp	Leu	Gly	Trp	Xaa	Asp	Trp	Xaa	Ile
	Ala	Pro	Xaa	Gly	Tyr	Xaa	Ala	Tyr	Tyr	Cys
20										
	Glu	Gly	Glu	Cys	Xaa	Phe	Pro	Leu	Xaa	Ser
	Xaa	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Xaa
25	Gln	Xaa	Leu	Val	His	Xaa	Xaa	Xaa	Pro	Xaa
	Xaa	Val	Pro	Lys	Xaa	Cys	Cys	Ala	Pro	Thr
	Xaa	Leu	Xaa	Ala	Xaa	Ser	Val	Leu	Tyr	Xaa
30										
	Asp	Xaa	Ser	Xaa	Asn	Val	Ile	Leu	Xaa	Lys
	Xaa	Arg	Asn	Met	Val	Val	Xaa	Ala	Cys	Gly
35	Cys	His,								

and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 = (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at res. 58 = (Asn or Lys);

Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res. 82 = (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 89 = (Lys or Arg);  
 5 Xaa at res. 91 = (Tyr or His); and Xaa at res. 97 = (Arg or Lys).

In another aspect, the invention comprises methods of using species of polypeptide chains comprising the conserved six cysteine skeleton plus additional cysteine residues identified in the OP2 proteins, and also comprising the conserved seven cysteine  
 10 skeleton plus additional cysteine residues identified in the OP2 proteins. The identification of renal therapeutic agents can thus be patterned after either of the following template amino acid sequence. The template sequences contemplated are SEQ ID NO: 3, described below (and also as Seq. No. 31 of U.S. Patent 5,266,683) comprising the conserved six cysteine skeleton plus the additional cysteine residue  
 15 identified in the OP2 proteins, and SEQ ID NO: 4, described below and as Seq No: 32 of U.S. Patent 5,266, 683), comprising the conserved seven cysteine skeleton plus the additional cysteine residue identified in the OP2 proteins. Each Xaa in these template sequences independently represents one of the 20 naturally-occurring L-isomer, alpha - amino acids, or a derivative thereof. Biosynthetic constructs patterned after this  
 20 template readily are constructed using conventional DNA synthesis or peptide synthesis techniques well known in the art.. Once constructed, renal therapeutic agents comprising these polypeptide chains can be tested as disclosed herein so that the agents define a polypeptide chain that have a conformation capable of being therapeutically effective when introduced into a subject with cystic kidney disease.

25

"Seven cysteine template" (SEQ ID NO: 3):

30	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Cys	Xaa
35	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa

	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
5	Cys	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
10	Xaa	Xaa	Xaa	Cys	Xaa	Cys	Xaa		

"Eight cysteine template" (SEQ ID NO: 4 comprising additional five residues at the N-terminus, including a conserved cysteine residue):

15	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
20	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys
	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa
	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
25	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Cys	Xaa	Xaa
30	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa
35	Cys	Xaa							

40 One preferred isolated renal therapeutic polypeptide comprises a polypeptide with an overall architecture and sequence identical to those sequences of SEQ ID NOS: 1-5. More preferably, the isolated polypeptide contains an amino acid sequence at least 60%, 80%, 90%, 95%, 98% or 99% homologous to an amino acid sequence from SEQ ID

NOS: 1-5. The isolated polypeptide is at least 5, 10, 20, 50, or 90 amino acids long. The isolated polypeptide includes at least 5, preferably at least 20, more preferably at least 50, most preferably at least 90 contiguous amino acids from SEQ ID NOS: 1-5.

The invention also pertains to an renal therapeutic polypeptide which includes an amino acid sequence described herein as well as other N-terminal or C-terminal amino acid sequences. For example, the polypeptide may include all or a fragment of an amino acid sequence from those in Table 1, including a portion such as SEQ ID NOS: 1-5 fused, in reading frame, to additional amino acid residues, preferably to residues encoded by genomic DNA 5' to the genomic DNA which encodes a sequence from those in Table 1. The renal therapeutic agent may also be a recombinant fusion protein having a first renal therapeutic portion and a second polypeptide portion having an amino acid sequence unrelated to the renal therapeutic polypeptide. The second polypeptide may be any of, for instance, glutathione-S-transferase, a DNA binding domain, or a polymerase activation domain. Preferably, the first renal therapeutic polypeptide includes amino acid residues from those in Table 1, including a portion such as SEQ IDS NOS: 1-5. The renal therapeutic polypeptide may also be a renal therapeutic fragment of a naturally occurring renal therapeutic agent which has biological activity.

### 20 **III. Production and Expression of Renal Therapeutic Polypeptides**

The renal therapeutic polypeptides described herein can be produced by any suitable method known in the art. Such methods include constructing a DNA sequence encoding renal therapeutic polypeptide sequences and expressing those sequences in a suitable transformed host. This method will produce isolated, renal therapeutic polypeptide. For example, cDNA may be isolated by screening a human cDNA library with a labeled DNA fragment encoding any one of SEQ ID NOS:1-5 and identifying positive clones by autoradiography. Further rounds of plaque purification and hybridization are performed using conventional methods.

However, the renal therapeutic polypeptide may also be produced, albeit less preferably, by chemical synthesis or a combination of chemical synthesis and recombinant DNA technology. In one embodiment of a recombinant method for

producing an renal therapeutic polypeptide, a DNA sequence is constructed by isolating or synthesizing a DNA sequence encoding wild type OP1. Optionally, the sequence may be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g., Zoeller et al., "Site-specific Mutagenesis Of The Human Fibroblast Interferon Gene", *Proc. Natl. Acad. Sci. USA*, 81, pp. 5662-66 (1984); United States Patent 4,588,585, incorporated herein by reference. Another method of constructing a DNA sequence encoding renal therapeutic polypeptide would be by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides may be preferably designed based on the amino acid sequence of the desired renal therapeutic polypeptide, and preferably selecting those codons that are favored in the host cell in which the recombinant renal therapeutic polypeptide will be produced.

The DNA sequence encoding renal therapeutic polypeptides, whether prepared by site directed mutagenesis, synthesis or other methods, may or may not also include DNA sequences that encode a signal sequence. The inclusion of a signal sequence depends on whether it is desired to secrete the renal therapeutic polypeptide from the recombinant cells in which it is made. If the cells chosen to provide the expression host are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence.

Standard methods may be applied to synthesize a polynucleotide sequence encoding a renal therapeutic polypeptide. For example, a complete amino acid sequence may be used to construct a back-translated gene. See Maniatis et al., *supra*. Further, a DNA oligomer containing a nucleotide sequence coding for the particular renal therapeutic polypeptide may be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide may be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Once assembled (by synthesis, site directed mutagenesis or another method), the mutant DNA sequences encoding a particular renal therapeutic polypeptide will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly may be confirmed by nucleotide sequencing, restriction mapping, and expression of a

biologically active polypeptide in a suitable host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

5 The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations may be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include  
10 known bacterial plasmids, such as plasmids from *E. coli*, including *coli* E1, pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single stranded DNA phages. Preferred *E. coli* vectors include pL vectors containing the lambda phage pL promoter (U.S. Patent 4,874,702), pET vectors containing the T7 polymerase promoter (Studier et al., **Methods in Enzymology** 185:  
15 60-89, 1990 ) and the pSP72 vector (Kaelin et al., supra). Useful vectors for insect cells include pVL 941. In addition, any of a wide variety of expression control sequences may be used in these vectors. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for  
20 example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the major operator and promoter regions of phage lambda, for example pL, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating system and other  
25 sequences known to control the expression of genes or prokaryotic or eukaryotic cells and their viruses, and various combinations thereof.

Any suitable host may be used to produce in quantity the renal therapeutic polypeptides described herein, including bacteria, fungi (including yeasts), plant, insect, mammal, or other appropriate animal cells or cell lines, as well as transgenic animals or  
30 plants. More particularly, these hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*,

fungi, yeast, insect cells such as *Spodoptera frugiperda* (SF9), and animal cells such as Chinese hamster ovary (CHO) and mouse cells such as NS/O, African green monkey cells such as COS 1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells, as well as plant cells in tissue culture. For animal cell expression, we prefer CHO cells and COS  
5 7 cells in cultures.

It should of course be understood that not all vectors and expression control sequences will function equally well to express a given renal therapeutic polypeptide. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control  
10 systems and hosts without undue experimentation. For example, to produce renal therapeutic polypeptide in large scale animal culture, the copy number of the expression vector must be controlled. Amplifiable vectors are well known in the art. See, for example, U.S. Patents 4,470,461 and 5,122,464; Kaufman and Sharp, *Mol. Cell. Biol.*,  
.....2: 1304-1319 (1982). The proteins produced by a transformed host can be purified  
15 according to any suitable method.

#### IV. Production of Fragments and Analogs

Fragments of a renal therapeutic protein can also be produced efficiently by recombinant methods, by proteolytic digestion, or by chemical synthesis, using methods  
20 known to those of skill in the art. In recombinant methods, internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes for the renal therapeutic polypeptide. Expression of the mutagenized DNA produces polypeptide fragments. Digestion with "end nibbling"  
25 endonucleases can also generate DNA's which encode an array of fragments. DNA's which encode fragments of a protein can also be generated by random shearing, restriction digestion or a combination.

Fragments can also be chemically synthesized using techniques known in the art such as the Merrifield solid phase f-Moc or t-Boc chemistry. For instance, peptides of  
30 the present invention may be arbitrarily divided into fragments of a desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length.

Examples of prior art methods which allow production and testing of fragments and analogs are discussed below. These, or analogous methods may be used to make and screen fragments and analogs of an renal therapeutic polypeptide which can be shown to bind cell surface receptor. Conversely, the methods can be used to make fragments and analogs of the cell surface receptor for a given renal therapeutic polypeptide of the invention.

#### V. Production of Altered DNA and Peptide Sequences: Random Methods

Amino acid sequence variants of a protein can be prepared by random mutagenesis of DNA which encodes a protein or a particular portion thereof. Useful methods include PCR mutagenesis and saturation mutagenesis. A library of random amino acid sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotide sequences.

PCR Mutagenesis: Briefly, *Taq* polymerase fidelity is used to introduce random mutations into a cloned fragment of DNA. See Leung et al., *Technique* 1:11-15 (1989). PCR conditions are chosen so that the fidelity of DNA synthesis is reduced by *Taq* DNA polymers using, for instance, a dGTP/dATP ratio of five and adding  $Mn^{+2}$  to the PCR reaction. The pool of amplified DNA fragments is inserted into appropriate cloning vectors to provide random mutant libraries.

Saturation Mutagenesis: The method is described generally in Mayers et al., *Science* 229: 242 (1985). Briefly, the technique includes generation of mutations by chemical treatment or irradiation of single stranded DNA in vitro, and synthesis of a cDNA strand. The mutation frequency is modulated by the severity of the treatment and essentially all possible base substitutions can be obtained.

Degenerate Oligonucleotide Mutagenesis: A library of homologous peptides can be generated from a set of degenerate oligonucleotide sequences. Chemical synthesis of degenerate sequences can be performed in an automatic DNA synthesizer, and the synthetic genes then ligated into an appropriate expression vector. See, Harang, S.A.,

*Tetrahedron* 39: 3 (1983); Itakura et al., **Recombinant DNA**, Proc. 3rd Cleveland Symposium on Macromolecules, pp. 273-289 (A.G. Walton, ed.), Elsevier, Amsterdam, 1981; Itakura et al., *Ann. Rev. Biochem.*, 53: 323 (1984).

5    **VI. Production of Altered DNA and Peptide Sequences: Directed Methods**

Non-random, or directed, mutagenesis provides specific sequences or mutations in specific portions of a polynucleotide sequence that encodes a renal therapeutic polypeptide, to provide variants which include deletions, insertion or substitutions or residues of the known amino acid sequence of the renal therapeutic polypeptide. The mutation sites may be modified individually or in series, for instance by: (i) substituting first with conserved amino acids and then with more radical choices depending on the results achieved; (ii) deleting the target residue; or (iii) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3.

Alanine scanning Mutagenesis: This method locates those residues or regions of a desired protein that are preferred locations or sites for mutagenesis. See Cunningham and Wells, *Science* 244: 1081-1085 (1989). In alanine screening, a residue or group of target residues are identified (e.g, charged residues such as Arg, Asp, His, Lys and Glu) and replaced by a neutral or a negatively charged amino acid such as alanine or polyalanine. This replacement can affect the interaction of the amino acids with the surrounding aqueous media in or outside the cell. Those having functional sensitivity to the substitutions are then refined by introducing further or other variants at, or for, the sites of substitution.

Oligonucleotide-Mediated Mutagenesis: This method may be used to prepare substitution, deletion, and insertion variants of DNA. See, Adelman et al., *DNA* 2: 183 (1983). Briefly, the desired DNA is altered by hybridizing an oligonucleotide primer encoding a DNA mutation to a DNA template which typically is the single stranded form of a plasmid or phage containing the unaltered or wild-type DNA sequence template of the desired protein (e.g., the Renal therapeutic peptide). After hybridization, a DNA polymerase is used to make an entire second cDNA of the template that will incorporate the oligonucleotide primer, and will code for the selected alteration in the

desired protein's DNA. Generally, oligonucleotide of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule.

5

Cassette Mutagenesis: This method (see Wells et al., *Gene*, 34: 315 (1985)) requires a plasmid or other vector that includes the protein subunit DNA to be mutated. The codon(s) in the protein subunit DNA are identified and there is inserted a unique restriction endonuclease site on each side of the identified mutation site(s), using the above-described oligonucleotide directed mutagenesis method. The plasmid is then cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard methods. This double-stranded oligonucleotide is the "cassette" and it has 3' and 5' ends that are compatible with the ends of the linearized plasmid so that it can be directly ligated therein. The plasmid now contains the mutated desired protein subunit DNA sequence.

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Combinatorial Mutagenesis: In this method (Ladner et al., WO 88/06630), the amino acid sequences for a group of homologs or other related proteins are aligned, preferably to promote the highest homology possible. All of the amino acids which appear at a given position of the aligned sequences can be selected to create a degenerate set of combinatorial sequences. The variegated library is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequence such that the degenerate set of potential sequences are expressible as individual peptides, or alternatively, as a set of larger fusion proteins contains the set of degenerate sequences.

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## 30 VII. Other Variants of Renal therapeutic Polypeptides

Included in the invention are methods for treating renal dysfunction using renal therapeutic molecules that are: allelic variants, natural mutants, induced mutants, proteins encoded by DNA that hybridize under high or low stringency conditions to a nucleic acid which encodes a polypeptide comprising a sequence such as SEQ ID NOS: 1-5 and polypeptides specifically bound by antisera to renal therapeutic peptides, especially to antisera to an active site or binding region of renal therapeutic.

The methods of the invention also feature uses of fragments, preferably biologically active fragments, or analogs of a renal therapeutic peptide. A biologically active fragment or analog is one having any in vivo or in vitro activity which is characteristic of the peptides shown herein or of other naturally occurring renal therapeutic peptide. Especially preferred are fragments which exist in vivo, such as fragments which arise from post-translational processing as the result of the removal of an amino-terminal signal sequences, as well as those made in expression systems such as in CHO cells. Useful renal therapeutic analogs possess biological activity. Most preferably, the fragment or analog has 10%, preferably 40%, or at least 90% of the activity of a renal therapeutic agent in any in vivo or in vitro assay. Analogs can differ from naturally occurring renal therapeutics in amino acid sequence or in ways that do not involve sequence, or both. Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatization as well as changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

Particularly preferred analogs include renal therapeutics or their biologically active fragments whose sequences differ from the wild type sequence by one or more conservative amino acid substitutions or by one or more non conservative amino acid substitutions, deletions or insertions which do not abolish the renal therapeutic biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine,

asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Other conservative substitutions can be taken from the table below:

5 TABLE 1: CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-ALa, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, Beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans 3,4 or 5-phenylproline, cis 3,4 or 5

		phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Generally, substitutions that may be expected to induce changes in the functional properties of renal therapeutic polypeptides are those in which: (i) a hydrophilic residue, e.g., serine or threonine, is substituted for (or by) a hydrophobic residue, e.g., leucine, isoleucine, phenylalanine, or alanine; (ii) a cysteine residue is substituted for (or by) any other residue; (iii) a residue having an electropositive side chain, e.g., lysine, arginine or histidine, is substituted for (or by) a residue having an electronegative charge, e.g., glutamic acid or aspartic acid; or (iv) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Other analogs used within the methods of the invention are those with modifications which increase peptide stability. Such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: analogs that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic analogs. Incorporation of D- instead of L-amino acids into the renal therapeutic polypeptide may increase its resistance to proteases. See, U.S. Patent 5,219,990 *supra*. The term "fragment", as applied to an renal therapeutic analog, will usually be at least about 20 residues, more typically at least about 40 residues, preferably at least about 60 residues in length. Fragments can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit renal therapeutic biological activity can be also assessed by methods known to those skilled in the art as described herein.

### VIII. Peptide Mimetics

The invention also provides for use of renal therapeutic agents that are peptide mimetics, i.e., peptide or non-peptide agents that have the same biological activity as isolated renal therapeutic agent.

One approach to generation of mimetics is initiated by determining the critical residues of a subject renal therapeutic polypeptide which are involved in molecular recognition of a receptor ligand. The residues are used to generate renal therapeutic-derived peptidomimetics which competitively or noncompetitively inhibit binding of

-37-

the renal therapeutic agent with its counterligand. For a similar approach, see generally, PCT Publication W0 90/04640 cDNAs encoding for the subunit of the high-affinity receptor for immunoglobulin E (3 May 1990).

In one method, scanning mutagenesis is used to map the amino acid residues of a particular renal therapeutic polypeptide involved in binding its cell surface receptor. Peptide mimetics are generated (e.g, diazepam or isoquinoline derivatives) which mimic those residues that bind to the receptor. For instance, non-hydrolyzable peptide analogs of such residues can be generated using non-peptides described in **Peptides: Chemistry and Biology** (G.R. Marshall ed.). ESCOM Publisher: Leiden, Netherlands, 1988) such as benzodiazepone (Freidinger et al.); azepine (Huffman et al.,); substituted gamma lactam rings (Garvey et al., id). Other mimetics include keto-methylene pseudopeptides (Ewenson et al., J. Med. Chem. 29: 295 (1986) and beta-amino alcohols (Gordon et al., Biochem. Biophys. Res. Comm. 126: 419 (1986). Another approach that does not require knowledge of the receptor is to use the renal therapeutic molecule itself to generate peptide mimetics using similar methods as described herein.

## IX. Methods of the Invention

The renal therapeutic agents of the present invention also may be tested in animal models of cystic kidney disease. See Section XI, below.

The renal therapeutic agents of the present invention may be evaluated for their therapeutic efficacy in causing a clinically significant improvement in a standard marker of renal function when administered to a human patient with or likely to develop cystic kidney disease. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na<sup>+</sup>), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like.

30

### A. Subjects for Treatment

As a general matter, the methods of the present invention may be utilized for any subject at risk of developing cystic kidney disease, or who has already been diagnosed as having clinically significant cystic kidney disease. The compound is useful in delaying onset of loss of renal function. Patients at risk of developing a disease of the invention may be treated periodically starting at the time of identification of their "at risk" status. For example, an infant with a genetic mutation, detected in utero by ultrasonography or by amniocentesis and chromosomal analysis, may be periodically treated from the time of birth, or even in utero, and continuing for life. A child or adult with pre-symptomatic disease might also be beneficially treated periodically for life.

Mammalian subjects which may be treated according to the methods of the invention include, but are not limited to, human subjects or patients. In addition, however, the invention may be employed in the treatment of domesticated mammals, including dogs, cats, horses, cattle, and laboratory animals. In addition, as a general matter, the subjects for treatment with the methods of the present invention need not present indications for treatment with the renal therapeutic agents of the invention other than those indications associated with risk of developing cystic kidney disease. That is, the subjects for treatment are expected to be otherwise free of indications for treatment with the renal therapeutic agents of the invention. In some number of cases, however, the subjects may present with other symptoms (e.g., osteodystrophy) for which treatment with the agents of the present invention would be indicated. In such cases, the treatment should be adjusted accordingly so to avoid excessive dosing.

One of ordinary skill in the medical or veterinary arts is trained to recognize subjects which may be at a substantial risk of cystic kidney disease. In particular, clinical and non-clinical trials, as well as accumulated experience, relating to the presently disclosed and other methods of treatment, are expected to inform the skilled practitioner in deciding whether a given subject is at risk of cystic kidney disease, or at risk of needing renal replacement therapy, and whether any particular treatment is best suited to the subject's needs, including treatment according to the present invention.

As a general matter, a mammalian subject may be regarded as being at risk of developing cystic kidney disease if the subject has been found to carry a genetic

mutation associated with a hereditary cystic kidney disease, or if the presence of such a gene is suggested by presence of hereditary cystic kidney disease in close relatives of the patient.

The success of a particular treatment may be measured by a variety of invasive and non-invasive procedures including biopsies, blood and urine chemical workup, MRI, CAT, ultrasonographic, or other imaging techniques.

Quite frequently, prognosis, diagnosis and/or treatment decisions are based upon clinical observations, such as the presence of a palpable abdominal mass, as well as by laboratory indications of renal function. One such indication of renal function is the glomerular flow rate (GFR), which can be measured directly by quantifying the rate of clearance of particular markers, or which may be inferred from indirect measurements.

#### B. Formulations and Methods of Treatment

Renal therapeutic agents in therapeutically effective amounts may be used in the form of pharmaceutically acceptable salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorasulfonate, cyclopentanepropionate, digluconate, dodecylsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroethanesulfonate, lactate, maleate, methanesulfone, 3-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts, such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine and so forth. Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl,

myristyl and stearyl chlorides, bromides and iodides, aralkyl halides such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The compounds described herein may be formulated into pharmaceutical compositions that may preferably be administered to a subject having, or at risk for, a renal dysfunction. Administration is systemic or local. Systemic administration may be done parenterally, or alternately by topical application, inhalation spray, nasally, buccally or via implanted reservoir. The term "parenteral" as used herein includes oral administration, subcutaneous, intravenous, intramuscular, intra-arterial, intra-synovial, intrasternal, intrahepatic, intrarenal, and intracranial injection or infusion techniques. Administration may be by periodic injections of a bolus of a renal therapeutic agent

Local administration (which may also include the above injection or infusion techniques) is preferably performed within the lumen at, or near, a site of renal injury or lesion. Persons having ordinary skill in the art will readily appreciate that a variety of medicament-dispensing devices, such as sustained release devices, grafts, catheters and stents (collectively referred to as "intra-luminal" devices) can be manufactured and used to locally deliver the compounds described herein. For example, U.S. Patent 5,464,450 (Nov. 7, 1995: "Biodegradable drug delivery vascular stent"- Buscemi, et al) describes a biodegradable stent that may comprise polylactic acid, polyglycolic acid (PGA), collagen or other connective proteins or natural materials, polycaprolactone, hyaluric acid, adhesive proteins, co-polymers of these materials as well as composites and combinations thereof and combinations of other biodegradable polymers. The stent itself therefore mediates slow local release of a drug into the lumen of a blood vessel. See, also, U.S. Patent 5,370,681 ("Polyluminal implantable organ"), U.S. Patent 5,328,470 ("...site-specific instillation of cells"). Such devices may be conveniently impregnated (i.e. surface coated and/or intersititally filled) with renal therapeutic agents described herein, and delivered locally to a site (i.e., a site of renal dysfunction) using methods available to persons having ordinary skill in the art. A preferred method of the present invention thus uses renal therapeutic polypeptide and polynucleotides at an local, intrarenal site of renal failure.

Other methods of the invention contemplate use of vesicles such as micelles or liposomes into, or onto which, is incorporated renal therapeutic agents. The vesicle may be a naturally occurring biological membrane, purified away from natural material or the vesicle may be a synthetic construction. Preferred vesicles are substantially  
5 spherical structures made of amphiphiles, e.g., surfactants or phospholipids. The lipids of these spherical vesicles are generally organized in the form of lipids having one or more structural layers, e.g., multilamellar vesicles (multiple onion-like shells of lipid bilayers which encompass an aqueous volume between the bilayers) or micelles. In particular, liposomes are small, spherical vesicles composed primarily of various types  
10 of lipids, phospholipids and secondary lipophilic components. These components are normally arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Typically, the polar end of a component lipid or lipid-like molecule is in contact with the surrounding solution, usually an aqueous solution, while the non-polar, hydrophobic end of the lipid or lipid-like molecule is in contact with the non-polar,  
15 hydrophobic end of another lipid or lipid-like molecule. The resulting bilayer membrane (i.e., vesicle) is selectively permeable to molecules of a certain size, hydrophobicity, shape, and net charge. Most vesicles are lipid or lipid-like in nature, although alternative liposome bilayer formulations, comprising a surfactant with either a lipid or a cholesterol exist.

20 Liposome vesicles may be particularly preferred in that they find many therapeutic, diagnostic, industrial and commercial applications. They are used to deliver molecules which are not readily soluble in water, or when directed timed release is desired. Because of their selective permeability to many chemical compounds, liposomes are useful as delivery vehicles for renal therapeutic agents. Upon reaching  
25 the target site, the liposomes may be degraded or they may fuse with the membranes of cells. Several methods of preparing vesicles such as liposomes are known. Among the more common of these are (1) sonication of a solution containing lipids sometimes followed by evaporation/lyophilization and rehydration (see, e.g. Stryer, **Biochemistry**, pp. 290-291, Freeman & Co., New York, (1988), and (55); (2) homogenization of lipid  
30 solution, sometimes at high pressure or high shearing force (see e.g. U.S. Pat. No. 4,743,449 issued 10 May 1988, and U.S. Pat. No. 4,753,788, issued 28 Jun. 1988), (3)

hydration and sometimes sonication of a dried film of vesicle-forming lipids wherein the lipid film is prepared by evaporation of a solution of lipids dissolved in an organic solvent (see e.g. U.S. Pat. No. 4,452,747 issued 5 Jun. 1984, U.S. Pat. No. 4,895,719 issued 23 Jan. 1990, and U.S. Pat. No. 4,946,787 issued 7 Aug. 1990), (4)

5 lyophilization or evaporation and rehydration (see e.g. U.S. Pat. No. 4,897,355 issued 30 Jan. 1990, EP 267,050 published 5 Nov. 1988, U.S. Pat. No. 4,776,991 issued 11 Oct. 1988, EP 172,007 published 19 Feb. 1986, and Australian patent application AU-A-48713/85 published 24 Apr. 1986), (5) solvent injection or infusion of a lipid solution into an aqueous medium or vice versa (see e.g. (56); U.S. Pat. No. 4,921,757  
10 issued 1 May 1990, U.S. Pat. No. 4,781,871 issued 1 Nov. 1988, WO 87/02396 published 24 Mar. 1988, and U.S. Pat. No. 4,895,452 issued 23 Jan. 1990), (6) spray drying (see e.g. Australian patent application AU-A-48713/85 published 24 Apr. 1986, and U.S. Pat. No. 4,830,858 issued 16 May 1989), (7) filtration (see e.g. WO 85/01161), (8) reverse-phase evaporation. See e.g. (57); and (9) combinations of the  
15 above methods. See e.g. (58) and (59).

Surfactants form micelles when added to aqueous solution above the surfactant's phase transition temperature. The surfactants may be composed of one or more aliphatic chains. These aliphatic chains may be saturated, unsaturated, or substituted in other ways, such as by ethoxylation; typically the aliphatic chain contains  
20 greater than about 12 carbons. Additional suitable surfactants include the following: lauryl-, myristyl-, linoleyl-, or stearyl- sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl, myristyl-, or cetyl- betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-,  
25 palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT series (Mona Industries, Inc., Paterson, N.J.).

Where the renal therapeutic agent is to be provided parenterally, such as by intravenous, subcutaneous, or intramuscular routes of administration, the agent  
30 preferably comprises part of an aqueous solution. The solution is physiologically acceptable (i.e., it is a physiological solution that is biocompatible) so that in addition to

delivery of the desired agent to the subject, the solution does not otherwise adversely affect the subject's electrolyte and/or volume balance. The aqueous medium for the agent thus may comprise normal physiologic saline (e.g., 9.85% NaCl, 0.15M, pH 7-7.4).

5       The compositions may be in the form of a sterile injectable preparation, for example a sterile injectable aqueous or oleaginous suspension. The suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents (see above discussion of vesicles). The sterile injectable preparation may also be a sterile injectable solution or a suspension in a non-  
10       toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be used including synthetic mono- or di-glycerides.  
15       Fatty acids such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils such as olive oil or castor oil, especially in the polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

      Pharmaceutical compositions comprise any of the above-described renal therapeutic  
20       compounds and homologs in therapeutically effective concentrations, together with any pharmaceutically acceptable carrier. The term "carrier" as used herein includes acceptable adjuvants and vehicles. Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins such as human serum albumin, buffers such  
25       as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes,  
30       polyethylene-polyoxypropylene-block polymers and wool fat.

Compositions containing renal therapeutic agents may also comprise one or more additional agents selected from the group consisting of corticosteroids, antiinflammatories (e.g., theophylline, sulfasalazine, aminosalicylates), antirheumatics, immunosuppressants (e.g., cyclosporin, rapamycin), antimetabolites (e.g., cyclophosphamide, methotrexate), immunomodulators (e.g., interferons). Further specific compounds within each of these classes may be selected from any of those listed under the appropriate group heading in **Comprehensive Medicinal Chemistry**, Pergamon Press, Oxford, England, pp. 970-986, incorporated herein by reference.

If desired, a given renal therapeutic agent or other agent may be made more soluble by association with a suitable molecule. For example, association of a mature OP/BMP dimer with an OP/BMP pro domain results in the pro form of the renal therapeutic agent which typically is more soluble or dispersible in physiological solutions than the corresponding mature form. In fact, endogenous members of the OP/BMP family are thought to be transported (e.g., secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of OP/BMP-secreting mammalian cells, e.g., cells transfected with nucleic acid encoding and competent to express the protein. Alternatively, a soluble species can be formulated by complexing the mature dimer (or an active fragment thereof) with a pro domain or a solubility-enhancing fragment thereof (described more fully below). Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Alternatively, the agents described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the renal therapeutic agents described herein typically are acid stable and protease-resistant (see, for example, U.S. Patent No. 4,968,590). In addition, at least one of these renal therapeutic agents, OP-1, has been identified in mammary gland extract, colostrum and 57-day

5 milk. Moreover, the OP-1 purified from mammary gland extract is therapeutically efficacious and also is detected in the bloodstream. Finally, soluble form OP-1, e.g., mature OP-1 associated with the pro domain, is therapeutically efficacious. These findings, as well as those disclosed in the examples below, indicate that oral and parenteral administration are viable means for administering the renal therapeutic agents of the invention to an individual. In addition, while the mature forms of certain renal therapeutic agents described herein typically are sparingly soluble, the form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, therapeutically efficacious form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

15 The compounds provided herein also may be associated with molecules capable of targeting the renal therapeutic agent to the desired tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on cells of the desired tissue, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Patent No. 5,091,513.

20 The amount of renal therapeutic agent as the active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, and the particular mode of administration. It should be understood, however, that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, the judgment of the treating physician and severity of the renal dysfunction. 25 The dosage and dose rate of the renal therapeutic agents described herein that are effective to prevent, suppress, or inhibit cystic kidney disease will depend on a variety of factors such as patient size, goal of treatment, specific pharmaceutical composition and judgment of the treating physician. As a general matter, daily or weekly dosage levels of between about 0.00001 and about 1000 mg are sufficient with, 0.0001-100 mg being 30 preferable and 0.001 to 10 mg being even more preferable. Alternatively, a daily or

weekly-dosage of 0.01-1000 ug/kg body weight, more preferably between about 10-700 ug/kg body weight, of active renal therapeutic agent are useful. Dosages can be administered continuously, but daily, multi-weekly, weekly or monthly dosages may also be employed. If used in a prophylactic context, dosages are given prior to clinical  
5 manifestations of the renal dysfunction at a level and frequency which will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, the judgment of the treating physician and susceptibility to the renal dysfunction.

10 As will be appreciated by one of ordinary skill in the art, the formulated compositions contain therapeutically effective amounts of the renal therapeutic agent. That is, they contain amounts which provide appropriate concentrations of the agent to the renal tissues for a time sufficient to prevent, inhibit, delay or alleviate permanent or progressive loss of renal function, or otherwise provide therapeutic efficacy. As will be  
15 appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition of the present invention will vary depending upon a number of factors, including the biological efficacy of the selected agent, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the formulation of the compound excipients, the administration route, and the treatment envisioned,  
20 including whether the active ingredient will be administered directly into a kidney or renal capsule, or whether it will be administered systemically. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the renal tissues, extent of renal function loss, and the overall health status of the particular subject. Dosages may be administered continuously, or daily, but it is currently  
25 preferred that dosages be administered once, twice or three times per week for as long as satisfactory response persists (as measured, for example, by stabilization and/or improvement of renal function by appropriate medical markers and/or quality of life indices) Less frequent dosages, for example monthly dosages, may also be employed. For subjects which would otherwise require continuous, bi-weekly or tri-weekly  
30 hemodialysis sessions, continuous, bi-weekly or tri-weekly intravenous or intraperitoneal infusions are not considered unduly inconvenient. In addition, in order

to facilitate frequent infusions, implantation of a semi-permanent stent (e.g., intravenous, intraperitoneal or intracapsular) may be advisable.

A general method of the invention involves administering to a subject (preferably a mammal such as a human) having, or at risk for, cystic kidney disease, a renal therapeutic agent selected from the group consisting of: (i) an isolated, renal  
5 therapeutic agent (e.g., protein, fragment, antibody, fusion); (ii) an isolated polynucleotide (e.g., a polynucleotide encoding an amino acid sequence comprising one of SEQ ID NOS: 1-5) capable of encoding, upon expression, a renal therapeutic agent ; and (iii) an isolated polynucleotide sequence capable of hybridizing under standard  
10 conditions to an isolated polynucleotide (e.g., a polynucleotide encoding an amino acid sequence comprising one of SEQ ID NOS: 1-5) capable of encoding, upon expression, a renal therapeutic agent.

As a general matter, the methods of the present invention may be used for any mammalian subject in, or at risk of, cystic kidney disease. Mammalian subjects include,  
15 but are not limited to humans since the methods may be employed in the treatment of domesticated mammals which are maintained as human companions (e.g., dogs, cats, horses), which have significant commercial value (e.g., dairy cows, beef cattle, sporting animals) or which have scientific value (e.g., captive or free specimens of endangered species). One of ordinary skill in the medical or veterinary arts is trained to recognize  
20 subjects which may be suitable for the methods of the invention. In particular, clinical and non-clinical trials, as well as accumulated experience, relating to the presently disclosed and other treatment methods, are expected to inform the skilled practitioner in deciding whether a given subject is in, or at risk of, renal dysfunction treatable using the present invention.

25

## **X. Gene Therapy**

Gene constructs can also be used as part of a gene therapy protocol to deliver nucleic acid encoding a form of renal therapeutic polypeptide. The invention features  
30 expression vectors for in vivo transfection and expression of renal therapeutic polypeptide in particular cell types so as to either: (i) antagonize the function of an renal

therapeutic molecule such as renal therapeutic in an environment (e.g., a renal dysfunction) in which that protein is misexpressed; (ii) agonize the function of a renal therapeutic agent. Expression constructs of renal therapeutic polypeptides may be administered in any biologically effective carrier that is capable of effectively delivery a polynucleotide sequence encoding the renal therapeutic polypeptide to cells in vivo. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly, plasmid DNA can be delivered with the help of, for example, cationic liposomes or derivatized (e.g., antibody conjugated) polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or  $\text{CaPO}_4$  precipitation carried out in vivo.

Any of the methods known in the art for the insertion of polynucleotide sequences into a vector may be used. See, for example, Sambrook et al., **Molecular Cloning: A Laboratory Manual**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and Ausubel et al., **Current Protocols in Molecular Biology**, J. Wiley & Sons, NY (1992), both of which are incorporated herein by reference. Conventional vectors consist of appropriate transcriptional/translational control signals operatively linked to the polynucleotide sequence for a particular renal therapeutic polynucleotide sequence. Promoters/enhancers may also be used to control expression of renal therapeutic polypeptide. Promoter activation may be tissue specific or inducible by a metabolic product or administered substance. Such promoters/enhancers include, but are not limited to, the native E2F promoter, the cytomegalovirus immediate-early promoter/enhancer (Karasuyama et al., *J. Exp. Med.*, 169: 13 (1989)); the human beta-actin promoter (Gunning et al., *Proc. Natl. Acad. Sci. USA*, 84: 4831 (1987)); the glucocorticoid-inducible promoter present in the mouse mammary tumor virus long terminal repeat (MMTV LTR) (Klessig et al., *Mol. Cell. Biol.*, 4: 1354 (1984)); the long terminal repeat sequences of Moloney murine leukemia virus (MuLV LTR) (Weiss et al., **RNA Tumor Viruses**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1985)); the SV40 early region promoter (Bernoist and Chambon, *Nature*, 290:304 (1981)); the promoter of the Rous sarcoma virus (RSV) (Yamamoto et al., *Cell*, 22:787

(1980)); the herpes simplex virus (HSV) thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA*, 78: 1441 (1981)); the adenovirus promoter (Yamada et al., *Proc. Natl. Acad. Sci. USA*, 82: 3567 (1985)).

Expression vectors compatible with mammalian host cells for use in gene therapy of  
5 tumor cells include, for example, plasmids; avian, murine and human retroviral vectors;  
adenovirus vectors; herpes viral vectors; and non-replicative pox viruses. In particular,  
replication-defective recombinant viruses can be generated in packaging cell lines that  
produce only replication-defective viruses. See **Current Protocols in Molecular  
Biology**: Sections 9.10-9.14 (Ausubel et al., eds.), Greene Publishing Associates, 1989.

10 Specific viral vectors for use in gene transfer systems are now well established.  
See for example: Madzak et al., *J. Gen. Virol.*, 73: 1533-36 (1992: papovavirus SV40);  
Berkner et al., *Curr. Top. Microbiol. Immunol.*, 158: 39-61 (1992: adenovirus); Moss et  
al., *Curr. Top. Microbiol. Immunol.*, 158: 25-38 (1992: vaccinia virus); Muzyczka,  
*Curr. Top. Microbiol. Immunol.*, 158: 97-123 (1992: adeno-associated virus);  
15 Margulskes, *Curr. Top. Microbiol. Immunol.*, 158: 67-93 (1992: herpes simplex virus  
(HSV) and Epstein-Barr virus (EBV)); Miller, *Curr. Top. Microbiol. Immunol.*, 158: 1-  
24 (1992:retrovirus); Brandyopadhyay et al., *Mol. Cell. Biol.*, 4: 749-754 (1984:  
retrovirus); Miller et al., *Nature*, 357: 455-450 (1992: retrovirus); Anderson, *Science*,  
256: 808-813 (1992:retrovirus), all of which are incorporated herein by reference.

20 Preferred vectors are DNA viruses that include adenoviruses (preferably Ad-2 or  
Ad-5 based vectors), herpes viruses (preferably herpes simplex virus based vectors),  
and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors,  
more preferably adeno-associated virus based vectors, most preferably AAV-2 based  
vectors). See, e.g., Ali et al., *Gene Therapy* 1: 367-384, 1994; U.S. Patent 4,797,368  
25 and 5,399,346 and discussion below.

The choice of a particular vector system for transferring, for instance, an Renal  
therapeutic polynucleotide sequence will depend on a variety of factors. One important  
factor is the nature of the target cell population. Although retroviral vectors have been  
extensively studied and used in a number of gene therapy applications, they are  
30 generally unsuited for infecting cells that are not dividing but may be useful in cancer  
therapy since they only integrate and express their genes in replicating cells. They are

useful for ex vivo approaches and are attractive in this regard due to their stable integration into the target cell genome.

Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a therapeutic or reporter transgene to a variety of cell types. The general  
5 adenoviruses types 2 and 5 (Ad2 and Ad5, respectively), which cause respiratory disease in humans, are currently being developed for gene therapy of Duchenne Muscular Dystrophy (DMD) and Cystic Fibrosis (CF). Both Ad2 and Ad5 belong to a subclass of adenovirus that are not associated with human malignancies. Adenovirus vectors are capable of providing extremely high levels of transgene delivery to virtually  
10 all cell types, regardless of the mitotic state. High titers ( $10^{13}$  plaque forming units/ml) of recombinant virus can be easily generated in 293 cells (an adenovirus-transformed, complementation human embryonic kidney cell line: ATCC CRL1573) and cryo-stored for extended periods without appreciable losses. The efficacy of this system in delivering a therapeutic transgene *in vivo* that complements a genetic imbalance has  
15 been demonstrated in animal models of various disorders. See Y. Watanabe, *Atherosclerosis*, 36: 261-268 (1986); K. Tanzawa et al, *FEBS Letters*, 118(1):81-84 (1980); J.L. Golasten et al, *New Engl.J. Med.*, 309 (11983): 288-296 (1983); S. Ishibashi et al, *J. Clin. Invest.*, 92: 883-893 (1993); and S. Ishibashi et al, *J. Clin. Invest.*, 93: 1889-1893 (1994), all of which are incorporated herein by reference.  
20 Indeed, recombinant replication defective adenovirus encoding a cDNA for the cystic fibrosis transmembrane regulator (CFTR) has been approved for use in at least two human CF clinical trials. See, e.g., J. Wilson, *Nature*, 365: 691-692 (Oct., 21, 1993). Further support of the safety of recombinant adenoviruses for gene therapy is the extensive experience of live adenovirus vaccines in human populations.

25 Adeno-associated viruses (AAV) have also been employed as vectors for somatic gene therapy. AAV is a small, single-stranded (ss) DNA virus with a simple genomic organization (4.7 kb) that makes it an ideal substrate for genetic engineering. Two open reading frames encode a series of *rep* and *cap* polypeptides. *Rep* polypeptides (*rep78*, *rep68*, *rep62* and *rep40*) are involved in replication, rescue and integration of the  
30 AAV genome. The *cap* proteins (VP1, VP2 and VP3) form the virion capsid. Flanking the *rep* and *cap* open reading frames at the 5' and 3' ends are 145 bp inverted terminal

repeats (ITRs), the first 125 bp of which are capable of forming Y- or T-shaped duplex structures. Of importance for the development of AAV vectors, the entire *rep* and *cap* s can be excised and replaced with a therapeutic or reporter transgene. See B.J. Carter, in **Handbook of Parvoviruses**, ed., P. Tijsser, CRC Press, pp. 155-168 (1990). It has  
5 been shown that the ITRs represent the minimal sequence required for replication, rescue, packaging, and integration of the AAV genome.

Adeno-associated viruses (AAV) have significant potential in gene therapy. The viral particles are very stable and recombinant AAVs (rAAV) have "drug-like" characteristics in that rAAV can be purified by pelleting or by CsCl gradient banding.  
10 They are heat stable and can be lyophilized to a powder and rehydrated to full activity. Their DNA stably integrates into host chromosomes so expression is long-term. Their host range is broad and AAV causes no known disease so that the recombinant vectors are non-toxic.

Furthermore, renal therapeutic polypeptides may also be introduced into a target  
15 cell using a variety of well-known methods that use non-viral based strategies that include electroporation, membrane fusion with liposomes, high velocity bombardment with DNA-coated microprojectiles, incubation with calcium-phosphate-DNA precipitate, DEAE-dextran mediated transfection, and direct micro-injection into single cells. For instance, an renal therapeutic polynucleotide encoding a renal therapeutic  
20 agent may be introduced into a endothelial cell by calcium phosphate coprecipitation (Pillicer et al., *Science*, 209: 1414-1422 (1980); mechanical microinjection and/or particle acceleration (Anderson et al., *Proc. Natl. Acad. Sci. USA*, 77: 5399-5403 (1980); liposome based DNA transfer (e.g., LIPOFECTIN-mediated transfection- Fefgner et al., *Proc. Natl. Acad. Sci. USA*, 84: 471-477 (1987), Gao and Huang,  
25 *Biochem. Biophys. Res. Comm.*, 179: 280-285, 1991); DEAE Dextran-mediated transfection; electroporation (U.S. Patent 4,956,288); or polylysine-based methods in which DNA is conjugated to deliver DNA preferentially to liver hepatocytes (Wolff et al., *Science*, 247: 465-468 (1990), Curiel et al., *Human Gene Therapy* 3: 147-154 (1992). Each of these methods is well represented in the art. Moreover, plasmids containing  
30 isolated polynucleotide sequences encoding renal therapeutic polypeptide may placed into, for example, endothelial cells using many of these same methods.

Renal therapeutic polypeptide itself may also be chemically modified to facilitate its delivery to a target cell. One such modification involves increasing the lipophilicity of the renal therapeutic polypeptide in order to increase cell surface binding and stimulate non-specific endocytosis of the polypeptide. A wide variety of lipopeptides, fatty acids, and basic polymers (e.g., tripalmitoyl-S-glycerylcysteil-serine; palmitic acid; polyarginine) may be linked to an renal therapeutic polypeptide to accomplish this. See U.S. Patent 5, 219,990, incorporated herein by reference.

Delivery may also be effected by using carrier moieties known to cross cell membranes. For example, an renal therapeutic agent that is a polypeptide such as one containing an amino acid sequence that includes SEQ ID NOS: 1-5 may be fused to a carrier moiety, preferably by genetic fusion, and the fused construct may be expressed in bacteria or yeast using standard techniques. Thus, polynucleotide sequences encoding Renal therapeutic polypeptide useful in the present invention, operatively linked to regulatory sequences, may be constructed and introduced into appropriate expression systems using conventional recombinant DNA techniques. The resulting fusion protein may then be purified and tested for its capacity to enter intact target cells and inhibit growth of the target cells once inside the target. For example, recombinant methods may be used to attach a carrier moiety to renal therapeutic polynucleotide sequences by joining the polynucleotide sequence encoding for Renal therapeutic polypeptide with the polynucleotide sequence encoding a carrier moiety and introducing the resulting construct into a cell capable of expressing the conjugate. Two separate sequences may be synthesized, either by recombinant means or chemically, and subsequently joined using known methods. The entire conjugate may be chemically synthesized as a single amino acid sequence.

Useful carrier moieties include, for example, bacterial hemolysins or "blending agents" such as alamethicin or sulfhydryl activated lysins. Other carrier moieties include cell entry components of bacterial toxins such as *Pseudomonas* exotoxin, tetanus toxin, ricin toxin and diphtheria toxin. Other useful carrier moieties include proteins which are viral receptors, cell receptors or cell ligands for specific receptors that are internalized and cross mammalian cell membranes via specific interaction with cell surface receptors. Such cell ligands include epidermal growth factor, fibroblast

growth factor, transferrin and platelet derived growth factor. The carrier moiety may also include bacterial immunogens, parasitic immunogens, viral immunogens, immunoglobulins, cytokines.

In a preferred embodiment, purified human immunodeficiency virus type-1 (HIV) tat protein is the carrier moiety. Purified human immunodeficiency virus type-1 (HIV) tat protein is taken up from the surrounding medium by human cells growing in culture. See Frankel et al., *Cell* 55: 1189-1193, (1988); Fawell et al., *Proc. Natl. Acad. Sci. USA*, 91: 664-668 (1994)( use of tat conjugate); and Pepinsky et al., *DNA and Cell Biology*, 13: 1011-1019 (1994)(use of tat genetic fusion construct), all of which are incorporated herein by reference. See also co-pending and commonly assigned PCT Application Serial Number PCT/US93/07833, published 3 March 1994, incorporated herein by reference, which describes the tat-mediated uptake of the papillomavirus E2 repressor; utilizing a fusion gene in which the HIV-1 tat gene is linked to the carboxy-terminal region of the E2 repressor open reading frame. The tat protein can deliver, for example, renal therapeutic polypeptide and polynucleotide sequences into cells, either in vitro or in vivo. For example, delivery can be carried out in vitro by adding a genetic fusion encoding an renal therapeutic-tat conjugate to cultured cells to produce cells that synthesize the tat conjugate or by combining a sample (e.g., blood, bone marrow, tumor cell) from an individual directly with the conjugate, under appropriate conditions. The target cells may be in vitro cells such as cultured animal cells, human cells or microorganisms. Delivery may be carried out in vivo by administering the renal therapeutic agent and tat protein to an individual in which it is to be used. The target may be in vivo cells, i.e., cells composing the organs or tissue of living animals or humans, or microorganisms found in living animals or humans. The ADP ribosylation from *Pseudomonas* exotoxin ("PE") and pancreatic ribonuclease have been conjugated to tat to confirm cytoplasmic delivery of a protein. The ADP phosphorylation is incapable of entering cells so that cytoplasmic delivery of this molecule would be confirmed if cell death occurs. Likewise, ribonuclease itself is incapable of entering cells so that inhibition of protein synthesis would be a hallmark of intracellular delivery using a tat conjugate.

Once introduced into a target cell, renal therapeutic polynucleotide sequences can be identified by conventional methods such as nucleic acid hybridization using probes comprising sequences that are homologous/complementary to the inserted renal therapeutic sequences of the vector. In another approach, the sequence(s) may be identified by the presence or absence of a "marker" gene function (e.g., thymidine kinase activity, antibiotic resistance, and the like) caused by introduction of the expression vector into the target cell. For instance, if a polynucleotide encoding a renal therapeutic agent is inserted into a vector having a dominant selectable marker gene such as a neomycin phosphotransferase gene under separate control of an SV40 early promoter, the sequence can be identified by the presence of the marker gene function (Geneticin resistance). Other methods of detecting the appropriate vector (e.g., PCR methods) will be readily available to persons having ordinary skill in the art.

In clinical settings, the delivery systems for the renal therapeutic polynucleotide sequence can be introduced into a patient by any number of methods, each of which is familiar to persons of ordinary skill. Specific incorporation of the delivery system in the target cells occurs primarily from specificity of transfection provided by the gene delivery vehicle, cell type or tissue type expression due to the transcriptional regulatory sequences controlling expression of the polynucleotide, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being localized by, for example, catheter (U.S. Patent 5,328,470) or stereotactic injection (Chen et al., *Proc. Natl. Acad. Sci. USA*, 91: 3054-3057 (1994)).

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is embedded. Where the complete gene delivery system can be produced intact from recombinant cells such as retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system. An effective amount of renal therapeutic polynucleotide sequence or polypeptide sequence may be administered intraocularly, parenterally, orally, intranasally, intravenously, intramuscularly, subcutaneously, or by other means. The term "pharmaceutically acceptable carrier" means one or more organic or inorganic

ingredients, natural or synthetic, with which the renal therapeutic molecule is combined to facilitate its application. A suitable carrier includes sterile saline although other aqueous and non-aqueous isotonic sterile solutions and sterile suspensions known to be pharmaceutically acceptable are known to those of ordinary skill in the art. In this regard, the term "carrier" encompasses liposomes and the HIV-1 tat protein (See Pepinsky et al., supra) as well as any plasmid and viral expression vectors. An "effective amount" refers to that amount which is capable of ameliorating or delaying progression of the diseased, degenerative or damaged condition. An effective amount can be determined on an individual basis and will be based, in part, on consideration of the symptoms to be treated and results sought. An effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In preferred methods, an effective amount of the renal therapeutic renal therapeutic polynucleotide sequence contained within its attendant vector (i.e., "carrier") may be directly administered to a target cell or tissue via direct injection with a needle or via a catheter or other delivery tube placed into the cell or tissue. Dosages will depend primarily on factors such as the condition being treated, the selected polynucleotide, the age, weight, and health of the subject, and may thus vary among subjects. An effective amount for a human subject is believed to be in the range of about 0.1 to about 50 ml of saline solution containing from about  $1 \times 10^7$  to about  $1 \times 10^{11}$  plaque forming units (pfu)/ml renal therapeutic renal therapeutic polynucleotide containing, viral expression vectors.

Target cells treated by renal therapeutic polynucleotide sequences (e.g., Renal therapeutic) may be administered topically, intraocularly, parenterally, intranasally, intratracheal, intrabronchially, intramuscularly, subcutaneously or by any other means. Target cells to be treated by renal therapeutic protein may be administered topically, intraocularly, parenterally, intranasally, intratracheal, intrabronchially, intramuscularly, subcutaneously or by any other means. The effect of a gene therapy vector carrying the renal therapeutic sequences can be determined by kidney biopsy or non-invasive kidney function tests such as described above.

## XI. Animal Models:

Appropriate animal models for cystic kidney disease are well known and well characterized. For instance, several mutations (either spontaneous or induced) in mice give rise to cystic kidney disease, and these have developed into the *cpk*, *pcy*, *jck*, and *cy* mutation models. In particular, cystic kidney in so-called "CFW<sub>wd</sub>" mice resembles human cystic kidney disease not only in terms of cyst localization and extrarenal manifestations but also with regard to onset of the disease and inheritance. See Schieren et al., *Nephrol. Dial. Transplant.*, 11 Suppl. 6: 38-45 (1996) and Aziz, *BioEssays* 17: 703-712 (1995). Furthermore, the Han:SPRD *cy*/+ rat model strikingly resembles human autosomal polycystic kidney disease. See Gretz et al., *Nephrol. Dial. Transplant.*, 11 Suppl. 6: 46-51 (1996).

Other cystic disease animal models are based on transgenic manipulations in which the *c-myc* gene or large T antigen is overexpressed and models in which cystic disease is induced by various chemicals such as corticoids. See Aziz, *supra*, Schieren et al., *supra*, and Sommardahl et al., *Pediatric Nephrology* 11: 728-733 (1997). Persons having ordinary skill in the art can conveniently test methods of treating cystic kidney disease in one or more of these animal models by assaying, for example, serum concentrations of alkaline phosphatase and alanine transaminase to assess liver damage, urine and serum osmolalities to assess renal concentrating ability and by performing various histochemical analyses of kidney cyst formation. See, for example, the methods described in Sommardahl et al., *supra*.

## Equivalents

While we have described a number of embodiments of this invention, it is apparent to persons having ordinary skill in the art that our basic embodiments may be altered to provide other embodiments that utilize the compositions and processes of this invention. Therefore, it will be appreciated that the scope of this invention includes all alternative embodiments and variations which are defined in the foregoing specification and by the claims appended hereto.

## SEQ ID NO: 2:

MetHisValArgSerLeuArgAlaAlaAlaProHisSerPheVal  
5 AlaLeuTrpAlaProLeuPheLeuLeuArgSerAlaLeuAlaAsp  
PheSerLeuAspAsnGluValHisSerSerPheIleHisArgArg  
10 LeuArgSerGlnGluArgArgGluMetGlnArgGluIleLeuSer  
IleLeuGlyLeuProHisArgProArgProHisLeuGlnGlyLys  
HisAsnSerAlaProMetPheMetLeuAspLeuTyrAsnAlaMet  
15 AlaValGluGluGlyGlyGlyProGlyGlyGlnGlyPheSerTyr  
ProTyrLysAlaValPheSerThrGlnGlyProProLeuAlaSer  
20 LeuGlnAspSerHisPheLeuThrAspAlaAspMetValMetSer  
PheValAsnLeuValGluHisAspLysGluPhePheHisProArg  
TyrHisHisArgGluPheArgPheAspLeuSerLysIleProGlu  
25 GlyGluAlaValThrAlaAlaGluPheArgIleTyrLysAspTyr  
IleArgGluArgPheAspAsnGluThrPheArgIleSerValTyr  
30 GlnValLeuGlnGluHisLeuGlyArgGluSerAspLeuPheLeu  
LeuAspSerArgThrLeuTrpAlaSerGluGluGlyTrpLeuVal  
PheAspIleThrAlaThrSerAsnHisTrpValValAsnProArg  
35 HisAsnLeuGlyLeuGlnLeuSerValGluThrLeuAspGlyGln  
SerIleAsnProLysLeuAlaGlyLeuIleGlyArgHisGlyPro  
40 GlnAsnLysGlnProPheMetValAlaPhePheLysAlaThrGlu  
ValHisPheArgSerIleArgSerThrGlySerLysGlnArgSer  
GlnAsnArgSerLysThrProLysAsnGlnGluAlaLeuArgMet  
45 AlaAsnValAlaGluAsnSerSerSerAspGlnArgGlnAlaCys

LysLysHisGluLeuTyrVal SerPheArgAspLeuGlyTrpGln  
AspTrpIleIleAlaProGluGlyTyrAlaAlaTyrTyrCysGlu  
5 GlyGluCysAlaPheProLeuAsnSerTyrMetAsnAlaThrAsn  
HisAlaIleValGlnThrLeuValHisPheIleAsnProGluThr  
10 ValProLysProCysCysAlaProThrGlnLeuAsnAlaIleSer  
ValLeuTyrPheAspAspSerSerAsnValIleLeuLysLysTyr  
ArgAsnMetValValArgAlaCysGlyCysHis  
15

We claim:

1. A method of treating cystic kidney disease comprising administering to a subject having, or at risk for, cystic kidney disease, an isolated, renal therapeutic agent or an isolated polynucleotide sequence encoding, upon expression, an isolated, renal therapeutic agent.  
5
2. The method of claim 1, wherein the isolated, renal therapeutic agent is selected from a member of an osteogenic protein/bone morphogenic protein (OP/BMP) family within a transforming growth factor-beta superfamily of proteins.  
10
3. The method of claim 2, wherein the isolated, renal therapeutic agent has at least 60% amino acid sequence homology with the group consisting of SEQ ID NOS: 1-5.
4. The method of claim 1, wherein the step of administering comprises  
15 administering an isolated polypeptide selected from the group consisting of those polypeptides listed in Table 1 and biologically active homologs thereof.
5. The method of claim 1, wherein the step of administering comprises systemic administration or local administration.  
20
6. The method of claim 5, wherein the step of local administration comprises administration with a device containing the renal therapeutic agent or polynucleotide sequence.
- 25 7. The method of claim 6, wherein the step of systemic administration comprises parenteral administration that is selected from the group consisting of subcutaneous, intravenous, intramuscular, intra-arterial, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intrarenal administration.
- 30 8. A method of renal therapy, comprising the step of administering to a subject having, or at risk for, cystic kidney disease, a therapeutically effective composition

selected from the group consisting of: (i) an isolated, renal therapeutic agent; (ii) an isolated polynucleotide capable of encoding, upon expression, an isolated renal therapeutic agent; and (iii) an isolated polynucleotide sequence capable of hybridizing under stringent conditions to the isolated polynucleotide sequence of (ii).

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9. The method of claim 8, wherein the step of administering comprises administering to a subject having, or at risk for, cystic kidney disease selected from the group consisting of autosomal recessive ("infantile") polycystic disease, multicystic dysplastic kidney disease, uremic medullary cystic disease, and autosomal dominant polycystic kidney disease.

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10. The method of claim 9, wherein the step of administering comprises systemic administration or local administration.

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11. The method of claim 10, wherein the step of local administration comprises administration with a device containing the therapeutically effective composition.

12. The method of claim 10, wherein the step of systemic administration comprises parenteral administration that is selected from the group consisting of subcutaneous, intravenous, intramuscular, intra-arterial, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intrarenal administration.

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13. The method of claim 8, wherein the isolated renal therapeutic agent is a polypeptide that is membrane bound.

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14. The method of claim 8, wherein the isolated renal therapeutic agent is a polypeptide that is soluble.

15. The method of claims 1 or 8, wherein the subject is human.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/09268

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-15  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/09268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9406449 A	31-03-1994	AT 165213 T	15-05-1998
		AT 162078 T	15-01-1998
		AU 678380 B	29-05-1997
		AU 4795193 A	03-03-1994
		AU 4797193 A	03-03-1994
		AU 4995593 A	03-03-1994
		AU 5129293 A	12-04-1994
		AU 5129393 A	12-04-1994
		AU 5162393 A	12-04-1994
		AU 5290893 A	12-04-1994
		AU 681362 B	28-08-1997
		AU 5590094 A	24-05-1994
		CA 2141554 A	17-02-1994
		CA 2141555 A	17-02-1994
		CA 2141556 A	17-02-1994
		CA 2144513 A	31-03-1994
		CA 2147598 A	11-05-1994
		DE 69316379 D	19-02-1998
		DE 69316379 T	30-07-1998
		DE 69318166 D	28-05-1998
		EP 0652953 A	17-05-1995
		EP 0653942 A	24-05-1995
		EP 0661933 A	12-07-1995
		EP 0665739 A	09-08-1995
		EP 0661987 A	12-07-1995
		EP 0680334 A	08-11-1995
		EP 0672064 A	20-09-1995
		ES 2114073 T	16-05-1998
		JP 7509611 T	26-10-1995
		JP 7509720 T	26-10-1995
		JP 7509721 T	26-10-1995
		JP 8501779 T	27-02-1996
		JP 8501558 T	20-02-1996
		JP 8501315 T	13-02-1996
		JP 8503198 T	09-04-1996
		WO 9403600 A	17-02-1994
		WO 9403075 A	17-02-1994
		WO 9403200 A	17-02-1994
		WO 9406447 A	31-03-1994
		WO 9406399 A	31-03-1994

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/09268

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/18 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	JENA N ET AL: "BMP7 null mutation in mice: developmental defects in skeleton, kidney, and eye." EXPERIMENTAL CELL RESEARCH, (1997 JAN 10) 230 (1) 28-37, XP002078233 see the whole document ---	1,2
Y	WO 94 06449 A (CREATIVE BIOMOLECULES, INC.) 31 March 1994 see the whole document ---	1-15
Y	EKBLOM P: "Genetics of kidney development." CURRENT OPINION IN NEPHROLOGY AND HYPERTENSION, (1996 MAY) 5 (3) 282-7. REF: 37, XP002078234 see the whole document ---	1-15

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

22 September 1998

Date of mailing of the international search report

08/10/1998

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# INTERNATIONAL SEARCH REPORT

ernational Application No  
PCT/US 98/09268

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 97 41881 A (CREATIVES BIOMOLECULES, INC.) 13 November 1997 see the whole document -----	1-15

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/09268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9406449 A		WO 9406420 A	31-03-1994
		WO 9410203 A	11-05-1994
		AU 681356 B	28-08-1997
		US 5652337 A	29-07-1997
		US 5652118 A	29-07-1997
WO 9741881 A	13-11-1997	AU 2832297 A	26-11-1997
		AU 2933997 A	26-11-1997
		WO 9741880 A	13-11-1997

